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SARS-CoV-2 S protein binding hACE2: viral entry, pathogenesis, prognosis, and potential therapeutic targets

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Abstract

Pneumonia cases of unknown etiology in Wuhan, China, were reported to the WHO on 31st of December 2019. Later the pathogen was reported to be a novel coronavirus designated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that causes Coronavirus Disease 2019 (COVID-19). SARS-CoV-2 is a novel pathogenic beta coronavirus that infects humans causing severe respiratory illness. However, multifarious factors can contribute to the susceptibility to COVID-19 related morbidity and mortality such as age, gender and underlying comorbidities. Importantly, SARS-CoV and SARS-CoV-2 entry into the host cells is mediated via ACE2 receptor. However, ACE2 receptor binding affinity to SARS-CoV-2 is 4 folds higher than that to SARS-CoV. Identification of different aspects such as binding affinity, differential antigenic profiles of spike glycoproteins, and ACE2 polymorphisms might influence the investigation of potential therapeutic strategies targeting SARS-CoV-2/ACE2 binding interface. Here we aim to elaborate on SARS-CoV-2 S1/ACE2 ligand that facilitates viral internalization as well as to highlight the differences between SARS-CoVs binding affinity to ACE2. We also discuss the possible immunogenic sequences of spike glycoprotein and the effect of ACE2 polymorphism on viral binding/infectivity and host susceptibility to disease. Furthermore, targeting of ACE2 will be discussed to understand its role in therapeutics.

Key words: COVID-19, SARS-CoV-2, ACE2 receptor, spike glycoprotein, ACE2 polymorphism, S glycoprotein immunogenic sequences.

1. Introduction

Idiopathic pneumonia cases in Wuhan, Hubei province, China were reported to the World Health Organization on 31st of December 2019 [1]. The pathogen was then reported to be a novel coronavirus designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes Corona virus disease 2019 (COVID-19) [1,2]. It was a severe blow to the world since the disease was transmitted like a wave all the way to the Americas from China. Therefore, WHO declared the COVID-19 outbreak a

"Public Health Emergency of International Concern" [2]. As of 7th of September 2020, 27,032,617 confirmed COVID-19 cases and 881,464 deaths were reported to the WHO [2].

The initial spread of the virus was linked to Huanan seafood market in Wuhan resulting in subsequent human to human transmission [3]. Bats were believed to be the natural host of SARS-CoV-2 virus that crossed the species barriers via an intermediate host to infect humans. It was thought that beside pangolins and snakes, turtles (Pelodiscus sinensis, Chrysemys picta bellii, and Chelonia mydas) are also believed to be a potential intermediate hosts of SARS-CoV-2 [4].

SARS-CoV-2 is the seventh discovered coronavirus to infect humans. Coronaviruses are members of the *Coronavirinae* subfamily that ramify from the family *Coronaviridae* (International Committee on Taxonomy of Viruses). *Coronavirinae* subfamily comprises 4 genera (*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*). Only 7 viruses of *Alphacoronavirus*, and *Betacoronavirus* genera infect humans causing respiratory illness. However, SARS-CoV, MERS-CoV, and SARS-CoV-2 are three tremendously pathogenic ß coronaviruses that cause severe respiratory syndrome in humans [5].

SARS-CoV-2 is commonly known to mediate entry into the host cells through the ACE2 receptor [6]. The ACE2 receptor is known to be expressed on the alveolar epithelial cells in the lungs. Binding of the viral spike protein to the ACE2 receptor leads to subsequent fusion of the viral envelope and host cell membrane thereby allowing successful viral entry into the host cells [7,8]. This binding of the virus to the ACE2 receptor leads to the endocytosis of lung alveolar epithelial cells causing irreversible damage to the lung tissue [9]. Following initial viral entry, cleavage between the S1 and S2 subunits of the viral glycoprotein takes places. This step is regulated by the receptor transmembrane protease serine 2 (TMPRSS2) that is a member of the Hepsin/TMPRSS subfamily [10]. Detachment of the S1 subunit from S2 causes the latter to undergo a conformational change that promotes and completes the fusion between the viral and host-cell membrane [11]. This fusion is a requisite for viral internalization, release of the viral content, replication and subsequent infection of other cells.

In this review we aim to focus on the structural binding mechanism of SARS-CoV-2 S1subunit binding to human ACE2 that facilitates viral internalization, possible immunogenic sequences of spike protein, effect of ACE2 polymorphism on viral binding and infectivity/ susceptibility to disease. Furthermore, targeting of ACE2 will be discussed to understand its role in therapeutics.

2. Structural binding of S1 protein to Human ACE2 receptor

ß Coronaviruses are enveloped viruses with four structural proteins known as Membrane glycoprotein (M), Spike protein (S), Nucleocapsid protein (N), and Envelope protein (E) [12]. Virion surface proteins such as the M, E, and S proteins in SARS-CoVs are well conserved [13,14]. It is thought that E and M proteins are responsible for viral entry, replication, and particles assembly within the host cells [15]. However, viral infection is initiated via binding of viral particles to cellular surface receptors of the host cells where Spike glycoprotein (S) of SARS-CoV-2 virus recognize and bind to human angiotensin converting enzyme 2 (hACE2) [13].

Spike protein priming is essential for host cell entry and virus infectivity of SARS-CoV-2. Host TMPRSS2 mediates priming of the trimeric S protein thus cleaving it into S1 and S2 subunits at the S1/S2 furin-like multibasic cleavage site that harbors multiple arginine residues indicating high cleavability [16,17]. The S1 subunit of the spike protein was found to be responsible for binding host cell receptor hACE2 while S2 subunit contributes to viral and cellular membranes fusion [18,19]. S1 subunit of the spike protein can be further divided in to two receptor binding sites one of which is the C terminal domain (CTD) and the N terminal domain (NTD). SARS-CoV-2 virus recognizes the hACE2 receptor via the CTD also known as receptor binding domain (RBD) [20].

Similar to other studied beta coronaviruses CTD structure shows two conserved subdomains [21]. One is the core subdomain that is composed of 5 inverse parallel beta strands (ß1, ß2, ß3, ß4, and ß7) structure with a disulfide bond between ß2 and ß4 strands. The other subdomain is a conserved extended insertion of (ß5 and ß6 strands, ∝4 and ∝5 helices, and loops) to the core subdomain forming the external

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subdomain that contains the receptor binding motif (RBM) which holds most of the binding residues of SARS-CoV-2 and hACE2 [13,22].

There are 3 pairs of cysteine residues (Cys336 – Cys361, Cys379 – Cys432, and Cys391 – Cys525) in the RBD forming disulfide bonds to help in stabilizing the ß sheet structure and 1 pair (Cys480 – Cys488) that facilitates connecting loops in the distal end of the RBD (Fig.1a) [22].

The C terminal receptor binding domain of SARS-CoV-2 S1 subunit binds the N terminal subdomain I of hACE2 [23]. The N terminal subdomain I of hACE2 has 2 lobes where the external subdomain of SARS-CoV-2 binds the small loop, with a concave structure of RBM accommodating the N terminal helix of the hACE2 [22].

Two lysine residues (31 and 353) on hACE2 are essential for binning to SARS-CoV-2 which are known as binding hotspots found in the SARS-CoV-2 / hACE2 interface. Hotspot Lys31 forms a salt bridge with Glu35 and hotspot Lys353 forming a salt bridge with Asp38. Both salt bridges are week due to the relatively long distance between both residues. However, the energy of salt bridges is enhanced when buried in a hydrophobic environment upon viral binding. This process is facilitated via hotspots interactions with adjacent RBD residues. The salt bridge in hotspot 31 breaks apart and each of Lys31 and Glu35 residues forms a hydrogen bond with Gln493 from SARS-CoV-2 RBM (Fig.1b). In addition, the Asn501 residue within the SARS-CoV-2 RBM forms a hydrogen bond to the main chain of RBM to slightly stabilize hotspot Lys353 with the presence of Lys353-Asp38 salt bridge (Fig.1c) [24]. A number of hydrophilic residues within SARS-CoV-2 / hACE2 interface were implicated in forming strong hydrogen bonds and salt bridge interactions. Those polar interlinkages include residue Ala475 of SARS-CoV-2-CTD interacting with Ser19 on hACE2, Asn487 with Gln24 (Fig.1d), Glu484 with Lys31 (Fig.1e), and Tyr453 with His34 (Fig.1f) [13,24]. The Phe486 and Tyr 489 of SARS-CoV-2 RBM interacts with Phe28, Leu79, Met82, and Tyr83 residues on hACE2 forming a hydrophobic pocket thus increasing the affinity of the virus to hACE2 receptor [13,22,24].

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The affinity between the ligand (SARS-CoV-2 S1) and the receptor (hACE2) reported in several studies by measuring the "dissociation constant K_d" [13,22]. Different dissociation constant values were reported due to variations in materials and methods used. K_d Values were ~15 nM, 44.2nM, 94.6 $\pm$ 6.5 nM, and 4.7 nM [13,22,24,25].

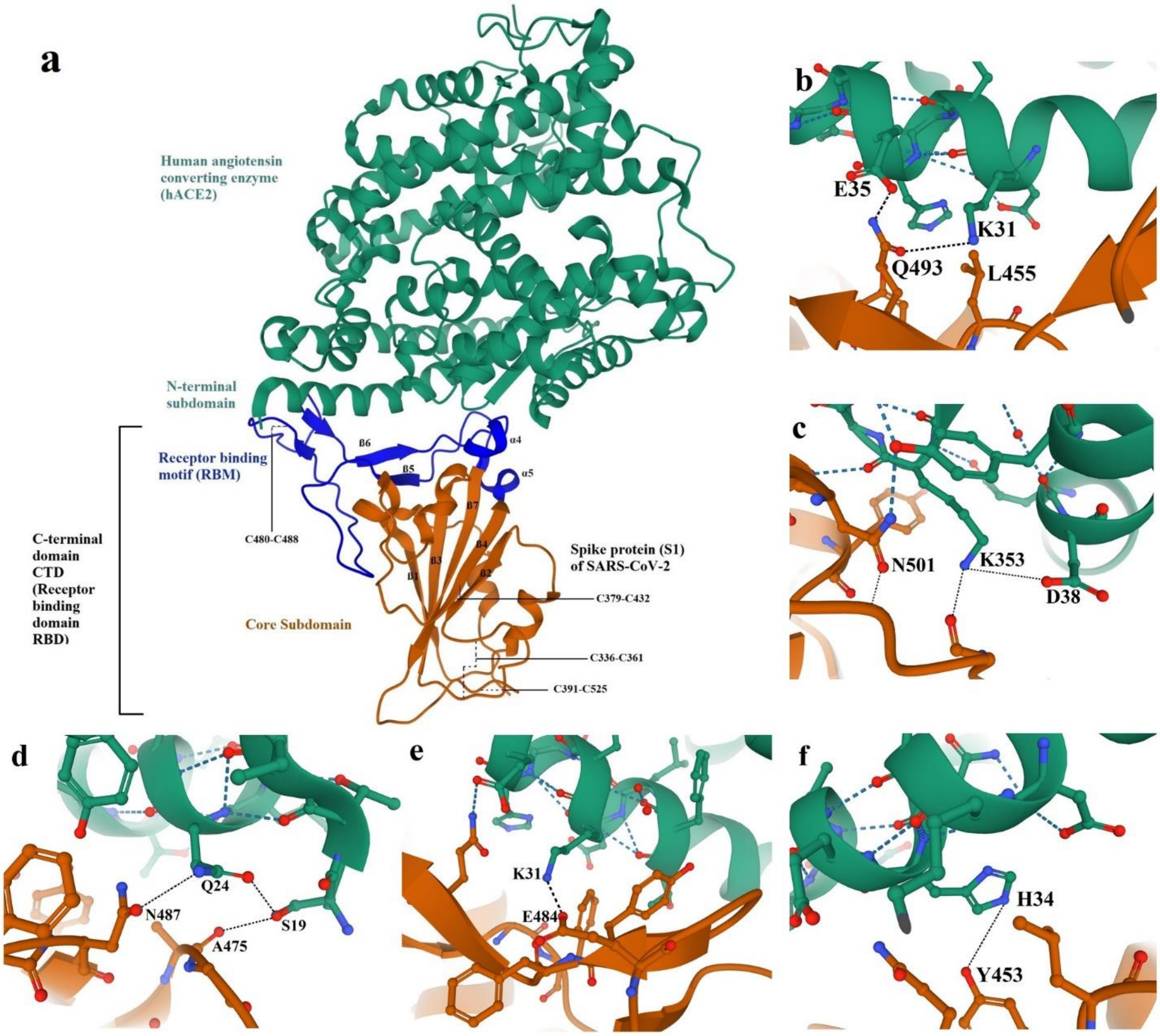

Figure 1: SARS-CoV-2/hACE2 structure and detailed binding: a. Overall SARS-CoV-2/hACE2 complex monomer. (b, c, d, e, and f) detailed structure of SARS-CoV-2 binding to human ACE2 receptor [13].

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3. Comparison between SARS-CoV-2 and SARS-CoV binding affinity to ACE2 receptor

Overall studies revealed approximately four folds higher binding affinity between SARS-CoV-2 S1 subunit and hACE2 compared with SARS-CoV/hACE2. Cryo electron microscopy and X-ray crystallography techniques facilitated the discovery of viral receptor binding domain/ human ACE2 complex structure [13,22,24]. Although, receptor binding mode was in general similar in both ligands; SARS-CoV-2 RBM forms more atomic interactions with hACE2 thus increasing the binding affinity compared to the ligand between SARS-CoV and ACE2 receptor. The ß5 and ß6 loop harbors the most variable region that facilitates stronger ionic and aromatic-aromatic interactions between SARS-CoV-2 and hACE2 compared to SARS-CoV loop [13].

When comparing the molecular interaction within the binding interface of the viral CTD, among the 24 residues of hACE2 that forms van der Waals forces (vdw) with residues of the virus CTD, 15 residues exhibit more contacts with SARS-CoV-2 CTD. Moreover, there are more residues on SARS-CoV-2 that binds hACE2 compared to SARS-CoV (21 versus 17) that can form vdw interlinks (288 versus 213) in addition to higher hydrogen (H) bonds interactions with SARS-CoV-2 than SARS-CoV (16 versus 11) [13]. The location that harbors the residues Leu455/Tyr442, SARS-CoV-2 Leu455 as SARS-CoV Tyr442 interact with the same residues on hACE2 Asp30, Lys31, and His34 (Fig. 2-a1) [13]. Hot spots Lys31 and Lys353 are critical in the binding of coronavirus RBD to hACE2. Lysine residues are stabilized where Asn501 on SARS-CoV-2 stabilizes hotspot 353/ Gln493 stabilize hotspot 31 which subsequently increase binding affinity of the novel virus (8). Asn501 of SARS-CoV-2 and Thr487 of SARS-CoV, both interact with Tyr41, Lys353, Gly354, and Asp355 residues in hACE2 receptor (Fig. 2-a2) [22].

Phenylalanine residue Phe 486 in SARS-CoV-2 RBD interacts with residues (Gln24, Leu79, Met82, and Tyr83) on hACE2 forming a hydrophobic pocket that increases the binding affinity while in SARS-CoV the corresponding residue is a leucine Leu472 side chain that provides a weaker interaction with (Leu79 and Met82) residues on hACE2 receptor (Fig. 2-a3) [13,22,24].

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Glutamine residue Gln493 in SARS-CoV-2 interacts with Glu35, Lys31, and His 34 on hACE2 and forms a hydrogen bond with Glu35 while in SARS-CoV corresponding residue Asn479 interacts only with His34 on hACE2 (Fig. 2-a4) [22].

Finally, there is a unique residue Lys417 harbored in an external location outside SARS-CoV-2 RBM that forms a salt bridge link with hACE2 Asp30 (Fig. 2b) [22].

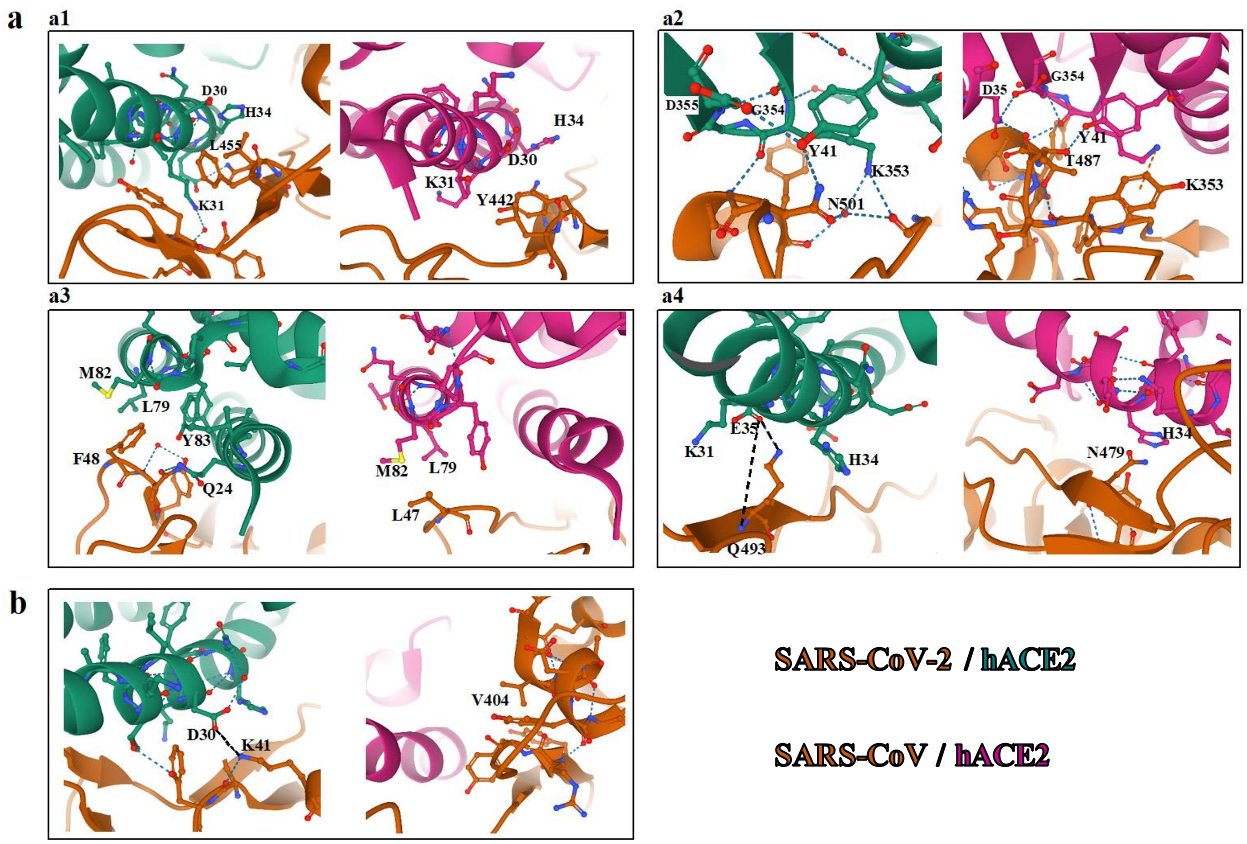

Fig.2: Comparison between SARS-CoV-2 and SARS-CoV binding hACE2 receptor. a. Interactions within the RBM of SARS-CoV-2 and SARS-CoV with hACE2 receptor. b. Variations in the K417/V404 position [13,26].

4. Differential antigenic profile in the spike glycoprotein between SARS-CoV-2 and SARS-CoV

Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein which plays a major role in the stimulation of the anti-viral immune response [27]. This S glycoprotein includes 1255 amino acids (aa) [GenBank submission ID: AAP13441.1] [28] in SARS-CoV and 1273 aa in SARS-CoV-2 [GenBank submission ID: QHD43416.1][29]. In addition, S glycoprotein comprises two functional subunits; S1 and S2 subunits, which are respectively responsible for the binding to the host cell ACE2 receptor and for the fusion of the viral and host cellular membranes. Indeed, the S1 subunit contains the Receptor Binding Domain (RBD) which mediates the virus-host cell interaction through binding to the cell's ACE2 receptor [19]. Interestingly, it has been demonstrated that SARS-CoV and SARS-CoV-2 S glycoproteins show 25.2% dissimilarity in the amino acids sequences [30]. In particular, a difference of 28% was observed at the Receptor Binding Domain residues [31]. Indeed, this RBD is located at the 318-569 aa sequence in SARS-CoV [GenBank submission ID: AAP13441.1][28] and at the 329-538 aa region in SARS-CoV-2 [GenBank submission ID: QHD43416.1][29]. This structural divergence between the two S glycoproteins might trigger changes in the antigenic properties of the two viruses leading to a differential modulation of the specific immune response.

Indeed, it has been demonstrated that most of the antibodies against SARS-CoV do not have a cross-neutralization activity toward SARS-CoV-2 [32]. Yet, the SARS-CoV viral epitopes targeted by the neutralizing antibodies are situated at the RBD [33]. Furthermore, a potent ACE2-blocking anti-SARS-CoV neutralizing antibodies showed limited cross-binding and cross-neutralizing activities against SARS-CoV-2 [30]. In addition, two monoclonal antibodies m396, CR3014, developed against SARS-CoV did not show evident binding to SARS-CoV-2 RBD [32]. Another monoclonal antibody CR3022 against SARS-CoV has shown cross-reactivity against the RBD region of SARS-CoV-2 [32]. Crystal structure and interaction studies were performed using CR3022 and the SARS-CoV-2 RBD in order to determine the cross-reactive epitope at the RBD region in SARS-CoV and SARS-CoV-2. Interestingly, the mapping of the interacting region has shown that out of 28 residues, 24 (86%) are conserved between SARS-CoV-2 and SARS-CoV. Thus, the presence of highly conserved sequences would explain the cross-reactivity of CR3022 between SARS-CoV-2 and SARS-CoV. However, *in vitro* binding affinity and micro-neutralization assays revealed that the antibody had higher affinity to SARS-CoV RBD and do not show any cross-neutralization activity between SARS-CoV-2 and SARS-CoV [34]. Interestingly, a recent study has identified a novel human monoclonal antibody called 47D11 that neutralizes SARS-CoV and SARS-CoV-2 *in vitro* [35]. However, the neutralization assay was not confirmed in vivo and the mechanism of anti-viral activity is not fully described, yet it seems to be independent from receptor-binding interference [35].

The antigenic difference between SARS-CoV and SARS-CoV-2 spike glycoproteins has also been confirmed using patients' serum. Interestingly, cross-reactivity of antibodies isolated from plasma samples of SARS-CoV and SARS-CoV-2 infected patients was mostly observed at the non-RBD regions and especially at the Spike protein ectodomain. Despite the presence of cross-reactivity in binding, cross-neutralization activity was not detected in any of the plasma samples [36]. For instance, the SARS-CoV-2 epitopes targeted by antibody repertoires remain uncharacterized. Therefore, the identification of SARS-CoV-2 immunogenic epitopes, that induce the secretion of specific neutralizing antibodies, will present an opportunity to develop targeted therapeutic strategies that would enhance the patients' clinical prognosis and contain the spread of the virus. Indeed, immune response to viral pathogens is essential to recovery and an effective serotherapy may be the best tool to prevent the spread of COVID-19. However, the development of specific assays for the evaluation of the quality and the quantity of viral specific antibodies and the characterization of neutralizing antibodies is very critical to provide a successful passive immunization protocol such as convalescent plasma therapy. Convalescent plasma therapy is an immunotherapeutic strategy which was successfully used for the prevention and treatment of various infectious diseases including MERS and SARS-CoV. Indeed, plasma from patients who have recovered from COVID-19 contains high titers of neutralizing antibodies which would be used as promising treatment option for COVID-19 rescue [37]. For instance, Mapping of antibodies epitopes based on computational biology enabled the identification of formulated sequence-based epitopes scores in spike proteins of SARS-CoV and SARS-CoV-2 [38]. It has been shown that SARS-CoV-2 had significantly higher antibody epitope score compared with SARS-CoV. Moreover, sequence alignment studies revealed that the non-conserved regions had significantly higher antibody epitope score as well as higher surface epitope accessibility confirming that these domains of the spike proteins are more antigenic and more available for antibody recognition [38]. In addition, it has been shown that SARS-CoV-2 RBD presents higher affinity towards the ACE2 receptor than that of SARS-CoV. This is linked to the presence of a furin-like cleavage site restricted to SARS-CoV-2. Altogether, different structural and functional characteristics could contribute to the increased infectivity of SARS-CoV-2 compared to SARS-CoV.

5. Implication of ACE2 mutations on the viral binding

The viral entry for SARS-CoV and SARS-CoV-2 is mediated through the interaction of the viral Receptor Binding Domain (RBD) with the ACE2 host cell membrane receptor [13]. The ACE2 gene is 40 kb long, contains 18 exons and is located at human X-chromosome [39]. ACE2 receptor is expressed by various human cells in the lung such as the airway and alveolar epithelial cells, the vascular endothelial cells and lung macrophages, as well as in brain cells and in cardiac, gastrointestinal and renal tissues [39].Therefore, all these cells could be a target for SARS-CoV-2. ACE2 gene is highly polymorphic yet the effect of ACE2 mutation on the interaction with SARS-CoV-2 S glycoprotein is still not fully described.

Computational based analysis helped to identify around 400 different types of mutations in ACE2 protein among different populations. These mutations would affect the virus-host interaction pattern and affinity and thereby potentially alter host susceptibility. Indeed, some human ACE2 variants including S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R are predicted to increase susceptibility to viral entry while other variants such as K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L and D509Y are predicted to decrease binding to SARS-CoV-2 S glycoprotein [40]. However, according to the available data, at patient level, the ACE2 polymorphism is not affecting the viral infectivity [41]. The full-length cDNA of the ACE2 gene in the lung was sequenced and 19 single nucleotide polymorphisms (SNPs) were discovered. This polymorphism was compared in 44 SARS-CoV cases, 16 anti-SARS-CoV antibody positive contacts, 87 antibody negative contacts, and 50 non-contacts in Vietnam and there was no evidence that these polymorphism at ACE2 gene level are involved in the disease prognosis [42]. Polymorphism of ACE2 gene was observed in different populations and was associated with abnormal regulation of blood pressure and increased risk to develop hypertension. ACE2 levels also seem to be upregulated in men compared to women and in Asian individuals compared to Caucasian, American and African populations. ACE2 differential gene expression could have an impact on the susceptibility to COVID-19 and on the prognosis of this viral infection [43].

6. Association between risk factors/ACE2 expression and susceptibility to SARS-COV-2

6.1. Chronic obstructive pulmonary disease patients (COPD):

ACE2 was found to be abundantly expressed on the surface of alveolar epithelial cells [44]. It was reported that gene and protein expression of ACE2 in epithelial cells of COPD patients were significantly higher in comparison with non-COPD individuals thus facilitating SARS-CoV-2 entry [45]. However, ACE2 receptor was found to be downregulated upon SARS-CoV-2 infection thus determining the severity of the disease according to the degree of ACE2 deficiency [11]. ACE2 downregulation would markedly facilitate the progression of inflammatory lesions and embolism in the lower respiratory tract [11]. COPD was found to be among the most prevailing comorbidities (1.5%, 95% CI: 0.9% - 2.1%) with an odds ratio OR of 2.46 (95% CI: 1.76 – 3.44) for disease severity in comparison to non- severe patients [46]. whereas the OR risk of death in severe cases patients with preexisting COPD was (1.49, 95% CI: 1.1 – 2.01) indicating that COPD might be considered as risk factor for disease severity and mortality [46,47].

6.2. Asthma

Patients with moderate to severe degree of asthma were categorized to be at high risk of developing severe SARS-CoV-2 and were likely to be hospitalized post SARS-CoV-2 infection where 17% of total hospitalized COVID-19 patients were of underlying asthma comorbidity [48,49]. This overrepresentation might be due to virus induced exacerbation of poor controlled asthma [50]. Immune response aberrations in asthmatic patients might be related to delayed and deficient innate anti-viral responses and lung interferons secretion (INF- α, INF-ß, and INF-λ) that contribute to asthma exacerbation [51-53]. Uncontrolled mild asthma patients when challenged with allergens, showed significant reduction in ACE 2 expression of the nasal and bronchial epithelia via interleukin 13 (IL-13) stimulation [54]. Another suggested mechanism for ACE 2 downregulation and shedding can be explained due to the viral-induced cytokine storm [55]. Downregulation of pulmonary ACE 2 expression thought to decrease the susceptibility of asthmatic patients to SARS-CoV-2 infection although that might also contribute to the loss of pulmonary function post COVID-19 [54,55].

It is an arena of controversy whether asthma medications pose any harm or they are of beneficial outcomes post SARS-CoV-2 infection in asthma patients. Azithromycin was found to reduce asthma attacks in adult patients by approximately 40% via reducing inflammation and increasing INF-ß / INF-λ production [56,57]. Azithromycin also showed suppression effect on viral replication in bronchial epithelial cells of asthmatic patients, thus preventing asthma exacerbation and severe lower respiratory tract illness [56,58].

Nebulizers are not recommended for COVID-19 patients due to its ability to deposit viral particles to the lower respiratory system as well as transmitting potentially viable virus via aerosols to susceptible nearby hosts [59,60].

Inhaled corticosteroids (ICS) used to treat persistent asthma and oral corticosteroids (OCI) used to treat acute lung exacerbation [61]. Corticosteroids have an antiviral activity represented by its ability not to inhibit IFNs secretion but attenuate viral-induced cytokines production [61]. On the other hand, deleterious effects were observed such as increased risk of pneumonia, increased urge for mechanical ventilation, increased viral shedding, and prolonged viral replication and viral clearance period [62-64] A number of biologics were approved for treating severe asthma such as Reslizumab, Omalizumab, Dupilumab, Mepolizumab, and Benralizumab for the purpose of reducing the frequency of exacerbation and to improve lung function [65]. Omalizumab, Reslizumab, Mepolizumab, and Benralizumab have a potential impact on viral shedding and viral clearance duration of Human rhinovirus HRV in asthmatic patients [63,66], While, Dupilumab decreases airway inflammation and asthma exacerbation [63]. However, it is important for asthma patients to continue their treatment regimens in order to control their symptoms as they might be confused with those of COVID-19 [67].

6.3. Cardiovascular Diseases

Based on the reports from China and Italy, the history of prevalent cardiovascular diseases was observed to be the most common co-morbidity in addition to diabetes and hypertension [68-70]. In a particular study 4% of the infected subjects were found to have pre-existing cardiovascular diseases [69]. An interesting fact to note here is that most hypertension and cardiovascular patients may suffer from ACE2 deficiencies due to deletions or inhibitions; as stated by different studies [71-73]. Indeed, ACE2 deficiency was found to enhance susceptibility to heart failures [71]. Also, heterozygote loss of ACE2 has been linked to an increased incidence of heart disease [74]. Based on all the above evidences it is possible to state that ACE2 deficiencies may play a pivotal role in the pathogenesis of SARS-CoV- 2 infections. Under these given conditions, one may speculate that mild or moderate ACE2 deficiencies may actually display a protective effect against the virus; however, contrastingly, this is highly unlikely due to the high affinity of SARS-CoV-2 to ACE2 receptors [16,19]. Instead, such deficiencies mainly noted in cardiovascular patients amplifies the imbalance between ACE2 and ACE leading to progression of inflammatory and hyper-coagulation processes that further worsens the prognosis of SARS-COV-2 infections [75].

6.4. Hypertension

It was reported that among 20982 patients diagnosed with SARS-CoV-2 infection, 12.6% were hypertension patients indicating that hypertension is one of the most prevalent comorbidities [76]. Moreover, the overall proportion of hypertension among 406 deceased people with SARS-CoV-2 infection was 39.7% of which approximately 81% subjects aged over 60 years old, thus pointing out age as a confounder [76].

Among hypertension treatments, ACE inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) are found to elevate the expression of hACE2 [77,78]. Theoretically, this might facilitate the SAR-CoV-2 uptake. However increased ACE2 expression and activity increases the conversion of angiotensin II (Ag II) to angiotensin 1-7 (Ang 1-7) protective anti-inflammatory peptide [79,80].

Pulmonary ACE2 was found to be downregulated upon SARS-CoV-2 infection, thus enhancing Ang II release [81,82]. Increased Ang II favors the Ang II / AT1R angiotensin II receptor 1 system over the ACE2 / Ang1-7 / mass system in the lung resulting in acute lung injury and subsequent ARDS Acute respiratory distress syndrome via AT1R [81]. Most importantly, available data up to date do not provide clear evidence whether hypertension or RAS blockers (ACEi and ARBs) favor the morbidity and/or the mortality of SARS-CoV-2 [83].

6.5. Chronic kidney disease

Chronic kidney disease (CKD) is a global health issue affecting 8-16% individuals worldwide [84,85]. Strikingly, recent studies indicate that the presence of kidney disease was associated with increased COVID-19 severity and related mortality [86-89]. Indeed, a meta-analysis shows that 83.93% of patients with CKD develop severe COVID-19 symptoms and that mortality was observed in 53.33% of the cases [90]. The involvement of kidney disease in COVID-19 prognosis and pathogenesis is likely to be multifactorial.

It has been reported that the ACE2 receptor is highly expressed in the kidney. Interestingly, RNA sequencing data from renal and respiratory tissues show that the ACE2 receptor expression in the kidney is 100-fold higher than that in the lung [91]. However, it is not clear whether SARS-CoV-2 replication occurs in these organs, possibly affecting their functional homeostasis. Indeed, a very recent study by Varga *et al.* confirmed by electron microscopy the presence of SARS-COV-2 viral particles in the kidney endothelial cells of a COVID-19 patient [92]. Moreover, Diao *et al.* found that SARS-COV-2 antigens were accumulated in kidney tubules [93]. Furthermore, the viral entry into renal epithelial cells suggests the possibility that the kidney could also become a viral reservoir leading to a viral persistence of SARS-COV-2 [94]. The above data demonstrate that the kidney is a specific target for SARS-COV-2 infection which can lead to an acute kidney injury (AKI) [95]. AKI can be a fatal complication of COVID-19 especially for patients with chronic kidney disease. Indeed, AKI has been reported in more than 20% of critically ill or deceased COVID-19 patients, a percentage that is consistent in studies from China [96], Italy [97] and United States [98]. Furthermore, the AKI may occur in COVID-19 patients due to many factors such as the cytopathic effect of SARS-COV-2 on kidney tissue, cytokine storm, organ crosstalk between lung and kidney or heart and kidney, deposition of immune complexes of viral antigen or virus-induced specific immunological effectors in the kidney [86,99]. All these aspects are profoundly interconnected and can contribute to kidney dysfunction. On the other hand, it is important to mention that dialysis patients are at high risk for COVID-19 related complications and death rate since they combine older age, malnutrition, cardiovascular disease, diabetes, lung disease and less efficient immune system [100].

6.6. Diabetes

It is not surprising that diabetes would promote an increase of COVID-19 severity since it has been shown already to be associated with a poor prognosis in other similar viral infections such as SARS-CoV [101] and *H1N1* infection [102]. Indeed, among 32 non-survivors from a group of 52 intensive care unit patients, diabetes was a predominant underlying comorbidity (22%) [96].

Diabetic patients are often treated with ACE inhibitors (ACEi) and angiotensin receptor blockers (ARB). Interestingly, these drugs contribute to a markedly increase of ACE2 expression as an adaptive response to restrain the high level of angiotensin I and II [103]. Consequently, these ACE2 stimulating drugs would probably increase the risk to SARS-COV-2.

On the other hand, innate immunity, the first line of defense against SARS-CoV-2, is compromised in patients with uncontrolled diabetes [104]. Indeed, it has been shown that chemotaxis and phagocytosis functions of neutrophils and monocytes are perturbed in diabetic individuals [105]. Moreover, it has been shown that pneumonia is an important cause of death in diabetic patients [106,107]. Therefore, one would suggest that the downregulation of the immune cells function in diabetic individuals would increase viral and bacterial infectious potential and promote bad prognosis in patients with COVID-19.

Furthermore, diabetes is characterized by an abundant production of inflammatory cytokines notably Interleukin 6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-α) in the absence of appropriate immuno-stimulation [108]. Indeed, it has been reported that the concentration of different inflammatory markers such as fibrinogen, C-reactive protein and D-dimer is more elevated in COVID-19 cases with diabetes compared to non-diabetic patients. This pro-inflammatory state makes diabetic people more susceptible to the cytokine storm which plays a major role in the development of acute respiratory distress syndrome leading to a rapid deterioration of the health condition of COVID-19 patients [109]. For instance, the elevated D-dimer level is also associated with a hyper-coagulation state that can lead to fatal thromboembolic complications and this pathology was reported as one of the major causes of death from COVID-19 infection [110,111].

Furthermore, the elevated levels of the enzyme furin observed in diabetic patients would probably promote the viral entry and replication [112]. Indeed, furin belongs the pro-protein convertase subtilisin/kexin family and it plays an important role in the entry of SARS-COV-2 in the host cell by cleaving the 2 subunits of the spike glycoprotein at a specific multibasic S1/S2 site [113,114]. However, the membrane fusion between the transmembrane subunit of spike protein S2 and the host cell membrane depends on S protein cleavage by the host cell protease furin at the S1/S2 site resulting in S protein activation and facilitate the entry into the host cell [115]. Interestingly, it has been reported that furin inhibitors can be considered as a treatment option for COVID-19 [16].

In addition, it has been reported recently that non-structural proteins of SARS-COV-2 attack the heme on the β-chain of hemoglobin leading to the disability of hemoglobin to carry oxygen [116]. Moreover, *Rimesh et al.* has suggested that SARS-CoV-2 might have a high binding affinity to glycated hemoglobin one [117]. Altogether, COVID-19 associated respiratory pathogenesis would be more complicated and life-threatening in diabetic individuals.

6.7. Obesity

Clinical reports from China [118], France [119] and the USA [120] suggested that obesity can be a risk factor for COVID-19 severity and related mortality due to the high prevalence of obese patients in critical condition. Indeed, a recent study has shown that 75.8% of the COVID-19 patients admitted in intensive care were obese and most of them required invasive mechanical ventilation [119]. Moreover, an epidemiologic analysis of 1,355 COVID-19 patients in USA found that at least 25% of the patients who died due to COVID-19 disease were overweight [120]. Multiple reasons can explain why obesity plays an important role in the pathogenesis of COVID-19. It has been reported that the expression level of ACE2 receptor is higher in the adipose tissue than in the lung tissue [121]. Therefore, having a more abundant adipose tissue than the normal population [122], obese individuals would possess a higher expression of ACE2 receptor and are consequently at a higher risk to SARS-CoV-2 infection [123]. In addition, once infected by SARS-COV-2, the adipose tissue can serve as a reservoir from which this virus could spread to other organs [124,125].

On the other hand, adipose tissue in obese individuals is characterized by a chronic inflammatory state and an abundant secretion of inflammatory cytokines such TNF-α, IL-6 and IL-1, and this would amplify the cytokine storm induced by SARS-CoV-2 infection and contribute to an aggravation of the COVID-19 disease in patients [124]. In addition, abdominal obesity is associated with impaired ventilation resulting in reduced blood oxygen saturation which would increase the COVID-19 disease complications [126].

6.8. Smoking

Cigarette smoking is considered today as a high-risk factor for many diseases due to the toxic, mutagenic, and carcinogenic properties of its components [127,128]. Recently, various studies have been carried out to identify the role of smoking in COVID-19 susceptibility and pathogenesis. Some of these studies suggest that smoking can be a risk factor for developing severe complications of COVID-19 [129-132]. Indeed, a recent meta-analysis study reports that smokers were 1.4 times more susceptible to severe symptoms of COVID-19 and approximately 2.4 times more likely to be admitted to intensive care unit and die compared to non-smokers [133].

Interestingly, a recent meta-analysis has revealed that pulmonary ACE2 expression was 25% higher in smokers compared to non-smokers [134]. Therefore, smoking might increase the risk of SARS-COV-2 infection via up-regulation of ACE2 receptor expression. Moreover, smoking has been shown to be detrimental for both innate (DCs, macrophages and NK cells) and adaptive immunity (regulatory T cells, CD8+ T cells, B cells and memory T/B lymphocytes) which make smokers more susceptible for bacterial and viral pulmonary infections [135,136]. Furthermore, smoking causes a decrease in pulmonary function [137]. Yet, it has been reported that smoking is the major cause of chronic obstructive pulmonary disease and chronic respiratory symptoms such as chronic cough, increased production of phlegm, wheezing, and breathing difficulties in healthy adults [138].

6.9. Elderly and Immuno-compromised Individuals

Increased age has also proven to be a factor that is associated with severe cases of SARS-CoV-2 infections [68-70]. The burden of the disease is much higher and among persons aged 70 years and above. In addition the mortality rates was found to be more than 20% among octogenarians [139]. In fact, Acute Respiratory Distress Syndrome (ARDS), the most common complication of SARS-CoV-2 infection was found to be more common in older patients [69]. Such a link between increasing age and severity of SARS-CoV-2 infections may be also attributed to the fact that ACE2 expression in the lungs decreases with increasing age [140]. Therefore, it may be speculated that ACE2 deficiencies in older individuals

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may also be one of the major reasons of increased severity of infection as in the case of cardiovascular patients. As a whole, ACE2 deficiencies are known to lead to the prolonged activation of the immune system progressing to inflammation. This establishes that a compromised immune system is at the center of SARS-CoV-2 infections; therefore immune-compromised individuals like aged individuals, cardiovascular patients and organ transplant recipients remain to be the most susceptible to the virus with comparatively worsened clinical prognosis.

6.10. Gender

Initial pandemic reports in China indicate that men are more susceptible to COVID-19 disease severity and related mortality than women [111,141,142]. Indeed, it has been shown that men represent 85% of the COVID-19 patients admitted in intensive care [111] and 70% of the patients who died of COVID-19 complications [143]. These findings can be explained by different biological differences between women and men. First, it has been confirmed that SARS-COV-2 uses the ACE2 receptor to invade the human cell and cause the final infection [9]. Interestingly, it has been reported that the density of ACE2 receptors in the reproductive organs is sex dependent. Indeed, the expression level of ACE2 receptors is much higher in testis than in the ovaries. Accordingly, a recent study suggests that the testis can serve as reservoir for SARS-COV-2 and that the testicular viral reservoirs play a crucial role in the viral persistence in men [144]. Furthermore, another study show that male reproductive systems are vulnerable to SARS-COV-2 infection and that COVID-19 patients show dramatic changes in sex hormones production due to the gonadal function impairment [145].

On the other hand, it has been reported that the X chromosome contains a high number of immune-related genes. Thus, women generally present a stronger innate and adaptive immune response and a faster clearance of pathogens than men [146]. Moreover, analysis of plasma samples from 331 COVID-19 patients has shown that the SARS-CoV-2 specific IgG antibody concentration in female patients tended to be higher than male patients in early phases of the disease [147]. The higher antibody concentration in female cases might play an important role in the clinical prognosis of COVID-19 disease. In the same context, it has also been reported that sex hormones play an important role in immune response regulation. Indeed, estrogen acts as an immune activator while testosterone acts as an immune suppressor [148].

Finally, recent studies suggest that vitamin D deficiency can be considered as a risk factor for respiratory viral infections [149]. Interestingly, it has been shown that vitamin D supplementation can prevent the viral acute respiratory infection [150] and decrease the inflammatory response to viral infections in airway epithelium [151]. Interestingly, a recent study has found that men are more susceptible to vitamin D deficiency than women [152]. Based on these findings, difference in vitamin D concentration between males and females could be involved in this sex-related susceptibility to COVID-19.

6.11. Pregnant women and neonates:

During the current pandemic of SARS-CoV-2 virus, the necessity to pay great attention to pregnant women population had urged. Pregnant women are a unique vulnerable group due to the anatomical, physiological, and immunological alterations that render them susceptible to infectious diseases. Progesterone and relaxants can pose several anatomical changes such as loosen the ribs ligaments and the diaphragm moves upwards as the pregnancy progresses. This will eventually lead to 20-30% decrease in “Functional Residual Capacity” (FRC) therefor resulting in hypoxia that is compensated by hyperventilation [153,154].

The fetus paternal antigens can provoke maternal immune system toward the fetus as a foreign body which isn’t the case in normal pregnancies where several complicated process result in fetal acceptance such as lymphopenia and decreased immune cells activity [155].

Pregnancy also significantly increases the expression of ACE2 receptor in several organs such as kidney, uterus, placenta, and umbilical cord which thought to modulate hemodynamics during gestation [156-158]. ACE2 is also highly expressed in the ovary, oocytes, and vagina [159]. All of the foregoing facts mentioned contribute to SARS-CoV-2 virus infectivity and morbidity [155,159].Notably, ACE2

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expression was found to be upregulated in heart, lung, and liver of human fetus which might pose severe pathological outcomes on neonates if infected by SARS CoV-2 virus [160].

Clinical manifestations appeared in SARS-CoV-2 pregnant patients did not differ from those appeared in non-pregnant patients [155,161], however, in some cases severe complications effected pregnant women and fetuses causing premature births, fetal distress, Premature rupture of fetal membrane, Cesarean section [159], and maternal acute renal impairment, renal dysfunction, and renal failure [161-163]. It was also observed that upon SARS-CoV-2 infection, ACE2 receptor was downregulated thus lowering the level of Angiotensin 1-7 which can mimic or worsen preeclampsia [164].

Infected newborns showed several symptoms such as fever, abnormal liver function, vomiting, tachycardia, thrombocytopenia, and shortness of breath [165].

Method of maternal-fetal transmission remained an issue of debate since some scientists claimed that vertical transmission through placenta and umbilical cord could explain the method of transmission [159,166,167]. On the other hand some studies reported that healthy newborns of infected mothers with Apgar score of 9-10 in 5 minutes [168]. Additionally, negative results of virus trace in placenta, amniotic fluid, and umbilical cord of infected pregnant women were reported [155,168].

Another possible route of transfection might be of paternal source through sexual contact with the pregnant women thus transmitting the virus via the semen of SARS-CoV-2 patients. Males' reproductive system is considered as viral reservoir even after recovery, virus might be detected in genital secretions [169].

6.12. Cancer patients and cancer therapies

Cancer patients are more susceptible to infections due to their poor health condition and immunosuppressive status caused by cancer and its therapies [170]. Consequently, active cancer patients infected with SARS-CoV-2 are more susceptible to severe clinical events compared to COVID-19 patients without cancer [171]. Therefore, COVID-19 patients with cancer had higher rates of suffering from at least one critical symptom, higher chances to be admitted to the ICU, higher possibility to utilize invasive mechanical ventilation, and increased death rates compared to non-cancer patients [170]. Moreover, COVID-19 Patients with hematologic cancers, lung cancer, and high stage metastatic cancers are at higher risk of developing severe clinical events in comparison to patients with other types of cancer, non-metastatic cancer patients, or those without cancer [170]. A study of viral pneumonia reported that mortality rates in cancer patients had a 24% mortality compared to 3% in non-cancer patients [172].

Several risk factors contribute to the deterioration of COVID-19 patients suffering from cancer, for instance, cytokine storm, and immune cells modulation [173]. SARS-CoV-2 infection elevates the levels of several cytokines such as IL-2, IL-6, IL-10, IL-8, and TNF-α. Furthermore, the Infection downregulates $CD4^{+}$ T cells, $CD8^{+}$ T cells, and B cells [173,174]. Acute inflammation is resolved as soon as the pathogen is cleared, however, if the pathogen persist then inflammation becomes chronic [174]. It is noteworthy that COVID-19 patients with cancer tend to have prolonged viral shedding and possible persisting infection sites which provoke chronic inflammation [171,174]. Chronic inflammation within a tissue can generate a pro-tumorigenic environment of immune cells and their secreted cytokines. This microenvironment is rich in reactive oxygen species (ROS) that can affect both infected and healthy cells thus causing DNA mutations. Secreted cytokines and metalloproteases facilitate vascular permeability and extracellular matrix proteins digestion respectively which finally result in tissue remodeling and cellular migration [174].

ACE2 is known to be an important regulator of lung function via protecting pulmonary tissue against injuries, therefore, its downregulation post SARS-CoV-2 infection can initiate severe immune responses and subsequent lung lesion. Reduced expression of ACE2 cause AT2 accumulation and AT1-7 dysregulation which consequently promotes inflammation. Indeed, ACE2 modulation is considered to be a major risk factor contributing to COVID-19 severity in cancer patients [175-177].

Cancer therapies might contribute to the severity of COVID-19 symptoms resulting in poor outcomes. For instance, chemotherapies might result in neutropenia, lymphopenia, and thrombocytopenia due to bone marrow damage caused, which in turn, render cancer patients more susceptible to infections. Furthermore, bone marrow transplant exerts the highest morbidity and mortality for the reason that this therapeutic approach eliminates the host immune system and replace it with that of his donor. On the other hand, conventional external beam radiation therapy might cause radiation-induced lymphopenia [171]. As to immunotherapies which include non-specific immunotherapies, cancer vaccines, chimeric antigen receptor T cell therapy, and immune-checkpoint inhibitors, that might exert their side effects via non-specific T cell activation against normal tissues [178]. Non-specific immunotherapies can cause anemia, thrombocytopenia, leucopenia, and vascular permeability that causes pulmonary edema and pleural effusion. On the other hand, cancer vaccines were believed to be correlated with minimal toxicity [171,178]. Chimeric antigen receptor T cell therapy can give rise to a serious side effect as cytokine syndrome [179]. Finally, immune checkpoint inhibitors are associated with immune-related adverse reactions that commonly affect gastrointestinal tract, skin, and endocrine glands. However, low incidence side effects might target cardiac system, and pulmonary system with rare chances of developing thrombocytopenia and pneumonitis [180].

7. Potential therapeutic targeting of Angiotensin-Converting enzyme 2

ACE-2, having a critical role in the entry, replication and pathogenicity of SARs-CoV-2, has been considered as a potential target for therapeutics. A number of clinical trials and pre-clinical studies focusing on various strategies including blocking of S protein binding to ACE-2 by soluble recombinant human ACE2 protein, blocking ACE-2 receptor by antibodies or small molecules, inhibition of transmembrane protease serine 2 (TMPRSS2) activities, utilizing ACE inhibitors and generation of spike based protein vaccine are underway worldwide (Table 1) [181].

Several studies have documented promising results targeting S protein binding of ACE-2 by using recombinant human ACE2 (rhACE2) [182-184]. *In vitro* studies with soluble rhACE2 in cell culture and engineered replicas of human blood vessels/kidneys in organoids have shown that rhACE2 forms high affinity binding with receptor-binding domain of SARS-CoV-2 [183]. This leads to neutralization of S protein on the SARS-CoV-2 surface thus reducing viral cellular entry and thereby protecting the host

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from developing severe ARDS or acute lung injury (ALI) facilitated by its peptidase-dependent function [182,183]. On the other hand, ACE2 antibodies or peptides that selectively block the interaction site of SARS-CoV-2 with ACE2 have also been identified as potential therapeutic candidates [13]. In this, it has been proposed that the generation of ACE2 fusion protein with extracellular domain of the ACE2 to a human immunoglobulin G Fc domain could be used to facilitate SARS-CoV-2 Spike protein binding to this protein, thus blocking viral entry and replication [185,186]. Furthermore, it has been suggested that if the effector function of Fc domain is, by some means, retained in the molecule, it could also allow recruitment of immune cells thus facilitating rapid activation of the host antiviral immune response and elimination of the virus. However, this ACE2-Fc strategy could have challenges such as mutating RNA virus may escape neutralization and increased levels of extracellular ACE2 could have implications in the host [187].

Inhibition of transmembrane protease serine 2 (TMPRSS2) activity is also considered an important strategy for blocking viral entry and replication [184]. Therefore, serine protease inhibitors of TMPRSS2 such as Camostat mesylate have been utilized in *in vitro* studies to study the effect on SARS-CoV-2 [188]. A recent study showed that Camostat mesylate significantly reduced the infection of Calu-3 lung cells by SARS-CoV-2 [16]. Previous studies based on SARS and MERS have also shown similar results indicating that inhibition of TMPRSS2 may facilitate reduction of infection of lung cells and therefore may be a suitable therapeutic target for SARS-CoV-2 [189,190]. Furthermore, development of synthetic inhibitors of TMPRSS2 can also be used to inhibit spike protein activation by TMPRSS2. However, limitations of this candidate is that molecular inhibitors of TMPRSS2 require validation of their specificity against other serine protease and need toxicological studies [191].

Utilizing ACE inhibitors (ACEIs/ARBs) is a topic of major interest for targeting of SARS-CoV-2. ACEIs/ARBs increase ACE2 activation subsequently leading to RAS deregulation. This deregulation may facilitate reduction in acute lung injury, heart injury, and renal damage induced by SARS-CoV-2 [123] However, clinical trials are on-going to provide evidence for the utility of ACE inhibitors for SARS-CoV-2.

Generation of spike1 subunit protein-based vaccine is considered a promising therapeutic target for various reasons [186,192]. Firstly, the structure of the SARS-CoV-2 S trimer in the pre-fusion conformation and the RBD domain in complex with ACE2 has been successfully determined thus making vaccine designing possible. Secondly, such a vaccine design it has already been tested for vaccine development against SARS-CoV and MERS-CoV with results that prove its applicability. Thirdly, it has the ability to bind to the ACE2 receptor which can help to target viral entry into the host cell. Therefore, because of its surface exposure, it is efficiently recognized by the host immune system to induce a robust anti-viral response [193].

Many studies are being published every day indicating various aspects related to therapeutic candidates against SARS-CoV-2. However, since COVID-19 is a new disease, any therapeutics targeting ACE-2 is deliberated until evidence of its efficacy via clinical trials sheds more light on its applicability.

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Table 1: On-going clinical trials focusing on ACE-2 in SARS-CoV-2 infection

Trial ID; Country	*Study title*	*Study type; Intervention*	*Enrolment eligibility*	*Primary expected outcome*
NCT04324996 China	A Phase I/II Study of Universal Off-the-shelf NKG2D-ACE2 CAR-NK Cells for therapy of COVID-19	Intervention; Intravenous infusion of constructed NKG2D-ACE2 CAR-NK cells secreting super IL15 superagonist and GM-CSF neutralizing scFv	Common, severe and critical pneumonia	Efficacy, safety and tolerability of treatment with NKG2D-ACE2 CAR-NK cells
NCT04328012 United States	Comparison of therapeutics for hospitalized patients Infected with SARS-CoV-2 (COVIDMED)	Randomized, double blind, Phase 2; Group 1: Losartan (Angiotensin II receptor blocker) Group2: Hydroxychloroquine Sulfate (anti-malarial) Group3: Lopinavir/ritonavir (antiretroviral) Group 4: Placebo	Hospitalized patients 72 hrs prior to randomization	Difference in National Institute of Allergy and Infectious Diseases COVID-19 Ordinal Severity Scale (NCOSS) between different treatment groups
NCT04355936 Argentina	Telmisartan for Treatment of COVID-19 Patients	Randomized, Open label, Phase 2; Group1:Telmisartan (Angiotensin II receptor blocker) plus standard care Group 2: standard care alone	PCR confirmed SARS-CoV-2 infection	Development of acute respiratory distress syndrome (blood oxygen saturation below 93 %) within 15 days of enrolment between different treatment groups
NCT04335786 Netherlands	Valsartan for Prevention of ARDS in Hospitalized Patients with SARS-COV-2 (COVID-19)	Randomized, double blind, Phase 4; Group1: Valsartan ((Angiotensin II receptor blocker) Group 2: Placebo	Hospitalized patients	Occurrence within 14 days of randomization of either: ICU admission;) mechanical ventilation or death
NCT04321096 Denmark	The Impact of Camostat Mesilate on COVID-19 Infection (CamoCO-19)	Randomized, Placebo controlled, Phase 2; Group 1: Camostat Mesilate (Serine protease inhibitor) Group 2: Placebo	Hospitalized patients 48 hrs prior to randomization	Days to clinical improvement from study enrolment
NCT04355026 Slovenia	Use of Bromhexine and Hydroxychloroquine for Treatment of COVID-19 Pneumonia	Randomized, Open label, Phase 4; Group1: Bromhexine (Serine protease inhibitor) plus hydroxychloroquine (anti-malarial) Group 2: hydroxychloroquine (anti-malarial) only	Hospitalized patients	Duration of hospitalization and disease between different treatment groups
NCT04329195 France	ACE Inhibitors or ARBs Discontinuation in Context of SARS-CoV-2 Pandemic (ACORES-2)	Randomized, Open label, Phase 3; Group 1: Discontinuation of RAS blocker (ACE inhibitor) therapy Group 2: Continuation of RAS blocker (ACE inhibitor)	Hospitalized patients chronically treated with RAS blockers prior to admission with a treatment duration ≥ 1 month	Time to clinical improvement from day 0 to day 28 between different treatment groups
NCT04348695 Spain	Study of Ruxolitinib Plus Simvastatin in the Prevention and Treatment of Respiratory Failure of COVID-19. (Ruxo-Sim-20)	Randomized, Open label, Phase 2; Group 1: Ruxolitinib plus simvastatin (ACE inhibitor) Group 2: Standard of care	Hospitalized patients with grade 3 or 4 of the WHO 7-point ordinal scale of severity categorization for COVID-19	Percentage of patients who develop severe respiratory failure (grade 5 or higher of the WHO 7-point ordinal scale of severity categorization) within 7 days of randomization.
NCT04337190 France	Impact of Angiotensin II Receptor Blockers treatment in Patients with COVID-19 (COVID-ARA2)	Observational; Blood sampling at the day of admission, day 3 and day 7	Intensive care unit admitted patients with ARDS	ACE2 level change over time
NCT04331574 Italy	Renin-Angiotensin System Inhibitors and COVID-19 (SARS-RAS)	Observational, Medical records; Verification whether chronic intake of RAS inhibitors modifies the prevalence and severity of the clinical manifestation of COVID-19.	Patients affected by COVID-19 refered to Italian outpatient clinics or hospitals	To determine whether antihypertensive ACE inhibitors or ARB increases the severity of the clinical manifestation of COVID-19

8. Conclusion

ACE2 has been identified as a key mediator of entry and subsequent manifestation of pathogenesis in SARS-CoV-2. Several factors such as binding affinity, differential antigenic profiles in spike glycoprotein, ACE2 mutations and risk factors that influence susceptibility to disease have been postulated and implicated in influencing expression and functional activity of ACE2. Furthermore, identification of these factors and their importance in investigating the therapeutic efficacy of ACE2 in SARS-CoV-2 have been highlighted in this review. It is concluded that further large-scale studies keeping these factors in perspective would allow better understanding/management of SARS-CoV-2. Of utmost importance is to focus on studies to understand the role of ACE2 with respect to variation in disease outcome. Stratification of patients with respect to ACE2 and the disease dynamics can then be utilized for personalized therapy with input of sophisticated clinical algorithms to be used for predictive modelling. This approach would allow identification of novel avenues for therapeutic modulation for COVID-19 and future viral diseases.

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